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The effect of corn on the utilization of L-tryptophan for pyridine nucleotide synthesis.

Reba King Coulter
University of Tennessee

To the Graduate Council:

I am submitting herewith a thesis written by Reba King Coulter entitled "The effect of corn on the utilization of L-tryptophan for pyridine nucleotide synthesis.." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Jane R. Savage, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

November 27, 1964

To the Graduate Council:

I am submitting a thesis written by Reba King Coulter entitled "The Effect of Corn on the Utilization of L-Tryptophan for Pyridine Nucleotide Synthesis." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Jane R. Savage
Major Professor

We have read this thesis and recommend its acceptance:

Bernadine Meyer

Nell P. Logan

Accepted for the Council:

Dean of the Graduate School

THE EFFECT OF CORN ON THE UTILIZATION OF L-TRYPTOPHAN FOR PYRIDINE NUCLEOTIDE SYNTHESIS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Reba King Coulter

December 1964

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CHAPTER I

INTRODUCTION

Pellagra was first associated with maize consumption in 1789, but its relationship to the disease was not understood at that time. Some researchers as early as 1924 hypothesized that pellagra might be caused by a deficiency of the amino acid tryptophan since this amino acid was missing from zein, the major protein in corn. However, it was not until 1945 that Krehl et al. (1,2) actually produced niacin deficiency symptoms and growth retardation in rats fed a niacin-deficient low-protein corn diet and reversed these ill effects by supplementing the diets with either niacin or tryptophan, thus showing a relationship between nicotinic acid and tryptophan.

Other investigators have shown that the addition of various proteins, such as zein and gelatin, or certain amino acids, or combinations of certain amino acids to a niacin-deficient low-protein diet depresses growth in rats and interferes with the utilization of tryptophan.

Further research by Goldsmith et al. (3,4) showed that clinical signs of pellagra could be produced in human subjects fed niacin-deficient low-protein corn or wheat diets and that these symptoms appeared more quickly in the subjects consuming the corn diets. When each of the diets was supplemented with either tryptophan or niacin the signs of pellagra disappeared.

Recent investigations by Belavady *et al.* (5) indicate the possibility that excess leucine may interfere in the synthesis of niacin from tryptophan by blocking the conversion of quinolinic acid to niacin. Since corn has long been associated with pellagra and since corn contains a high amount of leucine, the present study was made to investigate the effect of corn and zein on the utilization of L-tryptophan for pyridine nucleotide synthesis. The pyridine nucleotides are the metabolically active form of niacin in the body. In addition, an effort was made to determine the effect that various mixtures of amino acids as found in corn have on the utilization of L-tryptophan for pyridine nucleotide synthesis.

CHAPTER II

REVIEW OF LITERATURE

Pellagra-Nicotinic Acid Relationship

Pellagra victims are subject to dermatitis, inflamation of the tongue, diarrhea, pigmentation of the skin, and mental disorders. The disease has been known to exist among poverty stricken people in many areas of the world including the southern United States. For a number of years the cause of pellagra was not fully understood, but now that the disease may be prevented or cured by dietary means, it is no longer prevalent in any area of the United States. However, pellagra is still found in many other parts of the world.

Pellagra was first associated with the consumption of maize in 1789. The prevalence of the disease among populations subsisting largely on corn diets led many early researchers to believe that pellagra was caused by some toxic factor in maize (6). Others thought that the disease was caused by certain bacteria or other infectious agents. However, Voegtlin (7) and Goldberger and coworkers (8) demonstrated clearly that the diet of pellagrins was deficient in animal protein and that substantial intakes of animal protein prevented or cured the disease. Goldberger et al. (9) further showed that small amounts of yeast contained a pellagra-preventing substance and suggested that this substance was perhaps an unknown vitamin; this compound subsequently became known as the P-P (pellagra-preventing) vitamin.

Elvehjem et al. (10,11) in 1937 established the identity of the P-P vitamin when they cured canine black tongue, a disease in dogs comparable to pellagra in humans, by feeding nicotinic acid. Confirming Elvehjem's discoveries, Fouts et al. (12), Sydenstricker et al. (13), and Smith et al. (14) used either nicotinic acid or its amide successfully in curing pellagra in humans.

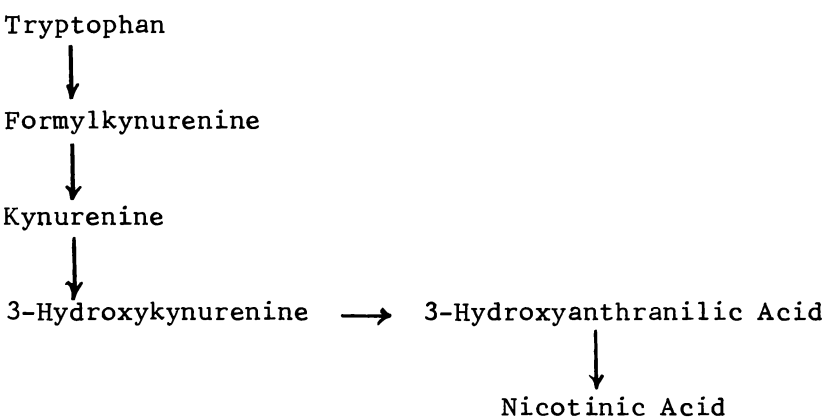
Tryptophan-Nicotinic Acid Relationship

Dann (15) observed that rats accumulated in their tissues and excreted more nicotinic acid in their urine and feces than could be accounted for by the dietary nicotinic acid intake. These results suggested that some precursor or precursors must be available for the synthesis of nicotinic acid.

Evidence was eventually furnished by Krehl et al. in 1945 (2) which showed that supplements of tryptophan as well as nicotinic acid reversed the deficiency symptoms and growth retardation of rats fed low-nicotinic acid corn diets. A further indication that tryptophan could act as a precursor of nicotinic acid was provided by Rosen et al. (16) when they discovered that the addition of tryptophan to the diet of rats fed nicotinic acid-deficient diets markedly increased the urinary excretion of N'-methylnicotinamide. Earlier, Huff and Perlzweig (17) had established that N'-methylnicotinamide was the major excretory product of nicotinic acid.

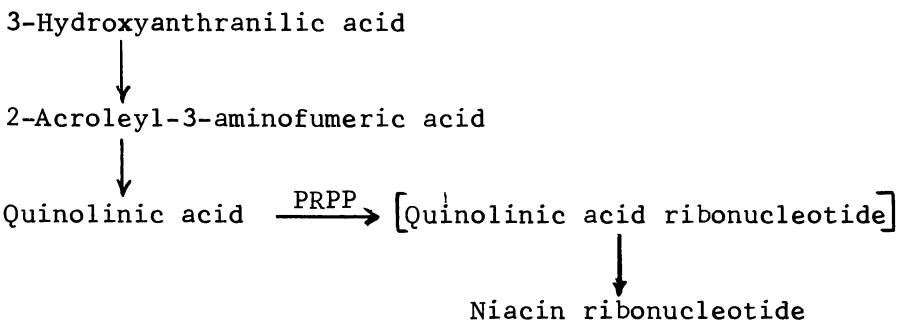
Although Krehl et al. (1,2), Rosen et al. (16), and other investigators successfully established the relationship of tryptophan

to niacin, it was not until 1949 that Heidelberger et al. (18) injected rats with radioactive DL-tryptophan-3-carbon-14 and found the radioactive carbon-14 label in the carboxyl carbon of nicotinic acid which had been isolated from the urine of the rats. This definitely established tryptophan as a precursor of nicotinic acid. Since then the work of many individuals has produced a fairly clear picture of the metabolic pathway by which tryptophan is converted to nicotinic acid. The intermediates in this pathway as outlined below have been isolated and identified. However, the mechanism by which 3-hydroxyanthranilic acid is converted to nicotinic acid has not been clarified until recently.



Recent work by Nishizuka and Hayaishi (19) has helped to clarify the last step in this pathway. These researchers prepared a rat liver supernatant solution and incubated it with 3-hydroxyanthranilic acid-carbon-14 uniformly labeled in the benzene ring. The major radioactive products recovered were niacin ribonucleotide and quinolinic acid. However, no carbon-14 label was found in either nicotinic acid or nicotinamide indicating that these compounds were not formed from 3-

hydroxyanthranilic acid as had been thought. Further work by these researchers demonstrated that 2-acroleyl-3-aminofumeric acid and quinolinic acid are intermediates in the conversion of 3-hydroxyanthranilic acid to niacin ribonucleotide. It was noted that the presence of 5-phosphoribosyl-1-pyrophosphate (PRPP) is essential for this conversion. As a result of this research, Nishizuka and Hayaishi postulated that the conversion of 3-hydroxyanthranilic acid to niacin ribonucleotide occurs in the following manner:



Pyridine Nucleotide* Synthesis

Nicotinamide adenine dinucleotide phosphate (NADP) was first isolated from horse erythrocytes by Warburg and Christian in 1935 (20) who identified nicotinamide as part of the coenzyme. Von Euler et al. (21) found nicotinamide to be a part of nicotinamide adenine dinucleotide (NAD). Based on much research including the studies made by Handler (22) and the recent findings by Nishizuka and Hayaishi (19) the following pathway for the synthesis of pyridine nucleotides from

*The term pyridine nucleotides in this thesis is used in the general sense to refer to a mixture of both oxidized and reduced nicotinamide adenine dinucleotide phosphate and nicotinamide adenine dinucleotide, the metabolic active forms of niacin in the body.

niacin ribonucleotide has been suggested.

$$\text{Niacin ribonucleotide} \xrightarrow{ATP^{*}} \text{Desamido-NAD} + PP_i$$

$$\text{Desamido-NAD} \xrightarrow[\text{Glutamine}]{ATP} \text{NAD} + \text{AMP} + \text{Glutamic acid} + PP_i$$

Numerous researchers have investigated the effects of tryptophan and niacin as precursors of pyridine nucleotides. The results of studies made by Williams et al. (23,24) on pyridine nucleotide synthesis in rats indicated that tryptophan played a greater role in pyridine nucleotide formation than did niacin. Feigelson et al. (25) demonstrated that the addition of equimolar amounts of tryptophan or niacin to the diet resulted in about the same level of pyridine nucleotide concentration in the liver. Chaloupka et al. (26) studied the effects of tryptophan or niacin supplements on the blood concentration of pyridine nucleotides in rats depleted of niacin or tryptophan. Results of her research indicated that at physiological levels tryptophan supported pyridine nucleotide formation more than niacin did.

Effect of Various Proteins or Amino Acids on the Tryptophan-Nicotinic Acid-Pyridine Nucleotide Relationships

Various proteins, such as zein and gelatin, and certain amino acids have been shown to affect the tryptophan-niacin relationship. Krehl et al. (1,2,27) produced growth depression and nicotinic acid

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*Abbreviations used: ATP, adenosine triphosphate; desamido-NAD, nicotinic acid adenine dinucleotide; PP_i, inorganic pyrophosphate; AMP, adenosine monophosphate.

deficiency symptoms in rats fed low-protein niacin-deficient diets to which either gelatin or 40% corn grits had been added. A less severe growth depression occurred in rats fed diets containing yellow or white corn in place of the corn grits. The ill effects caused by either of these diets could be prevented by feeding tryptophan or niacin.

Later Krehl et al. (27,28) substituted other cereals for the 40% corn grits and found that, unlike corn grits, grains such as whole rolled oats, polished rice, whole wheat flour, and soybean flour did not produce growth depression or niacin deficiency symptoms in the rat. The corn grits contained less tryptophan than did the other cereals and this may have been the reason for the growth difference. It was also suggested that the excessive amounts of certain amino acids in corn might cause an amino acid imbalance and thus prevent growth. Henderson et al. (29) and Hankes et al. (30) demonstrated that either single amino acids or mixtures of amino acids simulating proteins low in tryptophan when added to nicotinic acid-free diets retarded the growth of rats. Groschke et al. (31) fed a combination of proline, phenylalanine, alanine, leucine, and glutamic acid to rats and obtained a growth depression similar to that obtained with zein.

Hankes et al. (32) and Henderson et al. (33) found that a niacin-deficient 9% casein diet supplemented with threonine inhibited growth. Lyman and Elvehjem (34) were able to produce growth retardation and niacin deficiency symptoms in rats by feeding them a combination of L-arginine, DL-alanine, L-cystine, L-proline, DL-threonine, DL-phenyl-

alanine, and glycine. In each of these instances supplements of tryptophan to the diet reversed the growth depression.

The effect of gelatin and mixtures of the amino acids simulating gelatin on growth and pyridine nucleotide synthesis was investigated by Savage and Harper (35) in 1964. These researchers observed a marked growth depression in rats when 12% gelatin or a mixture of the indispensable amino acids simulating 12% gelatin was added to a low-protein niacin-deficient basal diet. The addition of tryptophan reversed the growth retardation in both cases. However, when a mixture of the dispensable amino acids simulating 12% gelatin was added to the basal diet, the growth depression thus produced could not be prevented by the addition of tryptophan.

The concentration of liver pyridine nucleotides increased significantly when either the basal diet or the diet containing the mixture of indispensable amino acids was supplemented with L-tryptophan. Only slight increases occurred in the pyridine nucleotide concentration of rats receiving the basal diet plus 12% gelatin or the basal diet plus a mixture of the dispensable amino acids when L-tryptophan was added. These results indicated that one or more of the dispensable amino acids of gelatin were responsible for the rats inability to utilize L-tryptophan for the synthesis of pyridine nucleotides. Further research indicated that the dispensable amino acids, L-4-hydroxyproline and glycine (amino acids in gelatin in the largest amounts) were responsible for this effect.

In human research, Goldsmith et al. (3,4) developed experimental pellagra in three subjects by feeding them corn diets containing 4.7 mg. niacin and 190 mg. tryptophan. The clinical signs of pellagra appeared after the subjects were on this regimen for 50 days. When the diets were supplemented with either nicotinamide or tryptophan all of the pellagra symptoms disappeared. At the same time one subject who was maintained for 122 days on a wheat diet containing 5.7 mg. of niacin and 230 mg. of tryptophan showed no clinical signs of pellagra.

Since the amounts of niacin and tryptophan in the corn diet were somewhat lower than they were in the wheat diet, Goldsmith et al. re-examined this problem in more detail by placing three subjects on a wheat diet containing 5 mg. of niacin and 200 mg. of tryptophan. These amounts were similar to the levels of niacin and tryptophan in the corn diet used earlier. In this instance, no clinical signs of pellagra occurred during an 80 day period on the wheat diet as compared to the appearance of pellagra symptoms after 50 days in subjects on a corn diet. From these results, Goldsmith and coworkers theorized that four possible reasons why pellagra symptoms appeared more quickly in subjects on the corn diet might be: (1) that corn contains a low amount of tryptophan, (2) that the niacin in corn may be in a form that cannot be used by the organism, (3) that large amounts of corn in a diet may cause an amino acid imbalance, and (4) that there may be a toxic substance in corn.

In tryptophan deficient proteins such as zein and gelatin, large amounts of one or more than one of the amino acids leucine,

glycine, or glutamic acid are found. The possibility that large amounts of an amino acid in a diet may cause an amino acid imbalance is supported by the recent research of Gopalan and Srikantia (36). They observed that pellagra is especially common in India among groups of people whose diet includes large quantities of Sorghum vulgar. Investigation of the amino acid content of Sorghum vulgar showed that the millet contained a high amount of leucine (12.9%) which is comparable to the amount of leucine in corn (14.9%). Belavady et al. (5) hypothesized that this excess leucine might in some way interfere in the conversion of tryptophan to niacin. To test this hypothesis, excess leucine was fed to both normal and pellagrous males. The urinary metabolites niacin, tryptophan, 5-hydroxyindoleacetic acid, indoleacetic acid, and indoxylsulfuric acid was measured. The only significant change found in normal males after the ingestion of leucine was a three fold increase in quinolinic acid excretion. However, in the pellagrins there was only a slight increase in the excretion of quinolinic acid, but tryptophan excretion after the ingestion of leucine fell from 113.5 mg. to 70.4 mg. per 24 hours. In further research on the control group, it was found that each time excess leucine was administered, there was a resulting rise in quinolinic acid excretion.

Since quinolinic acid is an important intermediate in the synthesis of nicotinic acid, these researchers believe that excess leucine may cause a block in the conversion of quinolinic acid into niacin.

Belavady *et al.* postulate that excessive amounts of leucine may act as a regulatory substance in the synthesis of pyridine nucleotides by inhibiting the reaction between 5-phosphoribosyl-1-pyrophosphate and quinolinic acid to form niacin ribonucleotide. In this case, an individual consuming large quantities of corn might be affected by both the low intake of tryptophan and a high intake of leucine.

CHAPTER III

EXPERIMENTAL PROCEDURE

Twenty male weanling albino rats of the Wistar strain ranging in weight from 36-48 grams were used in the first part of the study. The animals were divided into four groups consisting of five rats in each group. The rats were housed in individual metal cages except for two rats in each group which were housed together. The animals were weighed weekly during the six weeks experimental period. Rats were fed the various diets and distilled water ad libitum.

Diets

The basal diet was composed of (in %) vitamin-free casein, 6; fat-soluble vitamin mixture in corn oil, 5; choline, 0.15; DL-methionine, 0.3; niacin-free water-soluble vitamin mixture, 0.25; salt mixture, 5; (37) and sucrose, 83.3. All additions to the basal diet were made at the expense of sucrose. The composition of the water-soluble vitamin mixture is shown in Table I.

The fat-soluble vitamin mixture contained 272 mg. calciferol, 616 mg. Mazola corn oil, 8.5 g. of halibut liver oil, and 12.75 g. of α DL-tocopherol. Five grams of this fat-soluble vitamin mixture per 100 g. of ration provided 400 IU of vitamin A, 200 IU of vitamin D, and 10 mg. of vitamin E.

Two groups of rats (I and II) were fed the basal diet and two other groups (III and IV) were fed the basal diet containing 40%

TABLE I

WATER-SOLUBLE VITAMIN MIXTURE

Vitamin		Amount
		g.
Thiamine		0.250
Riboflavin		0.250
Calcium pantothenate		1.000
Pyridoxine HCl		0.130
Folic acid		0.010
Menadione		0.030
Biotin		0.005
Vitamin B_{12}	(1 g. triturate)	0.001
Inositol		5.000
Ascorbic acid		2.500

Sucrose was added to make a total weight of 125.00 g.

unenriched bolted Three Rivers Corn Meal (obtained locally). The diets of the rats in Groups II and IV were supplemented with 0.1% L-tryptophan.

Following the analysis of the first experiment, a second study was conducted with 55 male albino rats of the Wistar strain ranging in weight from 97-117 g. The animals were divided into eight groups consisting of 5-9 rats in each group. The average weights of all groups did not differ by more than one gram. The animals were housed in individual metal cages and were fed the various diets ad libitum for 14 days. The rats were weighed at the beginning and at the end of the experimental period.

A Kjeldahl analysis of the corn used in the first part of the study indicated the nitrogen content to be 1.37%. Using the general factor 6.25, the protein in 40 g. of corn was calculated to be 3.42%. On the assumption that one-half of the protein in corn is zein (38), the zein content of the corn was considered to be 1.71%.

Using the amino acid values for zein as reported by Block and Weiss (39), mixtures of the indispensable amino acids with and without leucine and mixtures of the dispensable amino acids which simulated those provided in 1.71% of zein were made. Only the L-isomers of the amino acids were used.

Two groups of rats (V and VI) were fed the basal diet plus 1.71% zein. Groups VII and VIII received the basal diet plus the complete mixture of indispensable amino acids simulating 1.71% of zein. Groups IX and X were fed the basal diet plus the indispensable

amino acids simulating 1.71% of zein without L-leucine, and Groups XI and XII were fed the basal diet plus the mixture of the dispensable amino acids simulating 1.71% of zein. One group of rats on each dietary regimen (Group VI, VIII, X, and XII) were supplemented with 0.1% L-tryptophan.

Extraction of Pyridine Nucleotides

At the end of the experimental period, the rats were decapitated and liver samples were removed as rapidly as possible. A sample ranging from 0.40-1.30 g. was weighed quickly on the O'Haus Balance and placed immediately into a homogenizing tube (previously heated in a boiling water bath) containing a 0.05 M. phosphate buffer at pH 5.4. NAD has been found to be stable at pH 5.4 while NADH is stable at pH 8.7 (40). The liver sample was incubated in the boiling water bath for one minute and then homogenized using a Potter-Elvehjem teflon pestle for approximately one minute. The tube containing the homogenate was temporarily stored in crushed ice while a second sample of liver was weighed and treated in the same manner as the first except that it was placed in a homogenizing tube containing a 0.05 M. phosphate buffer at pH 8.7.

After four animals had been sacrificed, one at a time, and the samples treated as described above, the liver homogenates obtained were centrifuged at 8,500 X g. for 15 minutes at 0° C. The supernatant fluid in the tubes containing pH 8.7 buffer was decanted and analyzed for NADH within 12 hours. The liver supernatant fluid for

the determination of NAD (pH 5.4 buffer) was decanted and frozen for analysis at a later date.

Analysis of NAD and NADH

The concentration of NAD and NADH in the liver supernatant fluids was determined spectrophotometrically at 340 mμ using the Beckman DU spectrophotometer according to the method described by Jedeikin and Weinhouse (40). At this wave length NADH absorbs light but NAD does not. In analyzing for NADH the optical density was read when the cuvette contained 0.3 ml. aliquot of liver supernatant fluid (if 0.1 ml. or 0.5 ml. aliquot of the supernatant fluid was used for analysis instead of 0.3 ml., the amount of water was increased or decreased accordingly), 1.5 ml. of pH 7, 0.1 M. KH_2PO_4, 1.0 ml. water, and 0.1 ml. acetaldehyde (10% solution). This first optical density reading was due to the presence of NADH plus whatever else, in the solution, also absorbed light at 340 mμ. A second optical density reading was obtained three minutes after the addition of 0.1 ml. of a 10% solution of yeast alcohol dehydrogenase (ADH) obtained from Worthington Biochemical Corporation. The optical density reading decreased because the NADH was converted to NAD. The following reaction occurred within the cuvette following the addition of ADH.

$$CH_3C(=O)-H + (\text{NADH in supernatant fluid}) \xrightarrow[\text{ADH}]{\text{yeast}} CH_3CH_2OH + NAD$$

The difference between the first and second optical density reading is a measure of the concentration of NADH in the aliquot analyzed. A third optical density reading was taken immediately following the

addition of another 0.1 ml. of ADH. The second addition of enzyme was necessary in order to correct for the dilution made by the first 0.1 ml. of ADH.

A similar procedure was used for the determination of NAD except that a 0.1 M. sodium pyrophosphate buffer, pH 9 was used instead of the 0.1 M. KH_2PO_4 buffer, pH 7, and 0.1 ml. of a 10% ethanol solution replaced 0.1 ml. acetaldehyde in the cuvette. The first optical density reading was low, because there was no NADH present. The second reading (made after the addition of 0.1 ml. of ADH) showed an increase as the NAD was converted to NADH. The following reaction occurred:

$$CH_3CH_2OH + (\text{NAD in supernatant fluid}) \xrightarrow[\text{ADH}]{\text{yeast}} CH_3C(=O)H + \text{NADH}$$

A third optical density reading was taken immediately following the addition of another 0.1 ml. of ADH. Again, the second addition of enzyme was necessary in order to correct for the dilution made by the first 0.1 ml. of ADH. From the change in optical density it was possible to calculate the concentration of NAD and NADH in the liver sample using the relationship that the change in one optical density unit is equivalent to 318 µg. of NAD or NADH.

Standard errors of the means were calculated for the weight gains per 7 days and for the liver pyridine nucleotide concentrations of all groups. The Student's "t" (41,42) test was used as the measurement of significance.

CHAPTER IV

RESULTS AND DISCUSSION

The effect of corn, zein, and mixtures of amino acids partially simulating zein with and without a 0.1% L-tryptophan supplement on the growth of rats is shown in Table II. In the groups with limited tryptophan, the addition of corn or the complete mixture of the indispensable amino acids to the niacin-free 6% casein diet increased the growth of rats fed these diets over that obtained with rats fed the basal diet, but the addition of zein to the basal diet markedly depressed growth. The increased growth of the rats fed the corn diet is in contrast to the results obtained by Krehl (1,2) who demonstrated that the addition of corn to a low-protein niacin-free diet produced growth depression and niacin deficiency symptoms in the rat. Krehl used 15% casein in his basal diet and replaced 40% of the entire ration with corn. Thus the protein content of Krehl's corn diet was reduced to 12.4%. However, in this research the corn diet contained 9.42% protein (6% casein plus 3.42% from corn) while the basal diet contained only 6% protein. The higher level of protein in the corn diets as compared to the basal diet may be responsible for these unexpected results. The increased growth of rats fed diets containing the complete mixture of indispensable amino acids partially simulating zein presumably was due to the better utilization and absorption of the free indispensable amino acids in contrast to

TABLE II

THE EFFECT OF CORN, ZEIN, AND MIXTURES OF AMINO ACIDS SIMULATING ZEIN WITH AND WITHOUT A 0.1% L-TRYPTOPHAN SUPPLEMENT ON THE GROWTH OF RATS

Groups	Diets	No. of Animals	Without Added Tryptophan g./7 days	No. of Animals	With 0.1% L-Tryptophan g./7 days
I and II	Basal	5	10 ± 1.4[a]	5	9 ± 0.2
III and IV	Corn	5	15 ± 0.3	5	21 ± 1.7[b]
V and VI	Zein	5	5 ± 0.7	5	8 ± 2.0
VII and VIII	Indispensable Amino Acids	8	17 ± 1.0	9	19 ± 1.0
IX and X	Indispensable Amino Acids Without Leucine	9	13 ± 0.6	9	24 ± 1.4[c]
XI and XII	Dispensable Amino Acids	5	10 ± 1.0	5	21 ± 1.4[d]

[a]Mean ± SE.

[b]Value within row greater than other value ($P < 0.02$).

[c]Value within row greater than other value ($P < 0.01$).

[d]Value within row greater than other value ($P < 0.02$).

those of the intact protein, zein. Normally, zein is a poorly digested protein. No significant growth difference was found between rats fed the mixture of indispensable amino acids without leucine and the basal diet. The addition of the dispensable amino acids simulating zein to the basal diet did not affect growth.

The growth depression which occurred in rats fed the zein diets was not relieved by the addition of 0.1% L-tryptophan. However, when a 0.1% L-tryptophan supplement was added to the diets containing corn, the indispensable amino acid mixture without leucine, and the dispensable amino acid mixture significant increases ($P < 0.02$, $P < 0.01$, and $P < 0.02$ respectively) in growth occurred. The greatest growth increase occurred in rats fed the mixture of indispensable amino acids without leucine. The addition of a 0.1% L-tryptophan supplement did not significantly change the growth of rats fed either the basal diet or the diet containing the complete mixture of the indispensable amino acids.

The effect of corn, zein, and mixtures of amino acids partially simulating zein with and without a tryptophan supplement on the total liver pyridine nucleotide concentration of rats is shown in Table III. The total pyridine nucleotide concentration was obtained by adding the NAD concentration to the NADH concentration. When no tryptophan supplement was fed, the addition of corn, zein, the complete mixture of the indispensable amino acids simulating zein, and the dispensable amino acids simulating zein to the basal diet did not affect the pyridine nucleotide concentration of the rats fed these diets. However, rats fed the diet in which leucine was omitted from the mixture

TABLE III

THE EFFECT OF CORN, ZEIN, AND MIXTURES OF AMINO ACIDS SIMULATING ZEIN WITH AND WITHOUT A 0.1% L-TRYPTOPHAN SUPPLEMENT ON TOTAL LIVER PYRIDINE NUCLEOTIDE CONCENTRATION OF RATS

Groups	Diets	No. of Animals	Without Added Tryptophan	No. of Animals	With 0.1% L-Tryptophan
			µg./g.		µg./g.
I and II	Basal	5	658 ± 70[a]	5	880 ± 91[b]
III and IV	Corn	5	691 ± 117	5	731 ± 109
V and VI	Zein	5	645 ± 26	5	722 ± 56
VII and VIII	Indispensable Amino Acids	8	676 ± 44	9	856 ± 27[c]
IX and X	Indispensable Amino Acids Without Leucine	9	979 ± 23[d]	9	1209 ± 58[c,e]
XI and XII	Dispensable Amino Acids	5	741 ± 53	5	790 ± 35

[a]Mean ± SE.

[b]Value within row different than other value ($P < 0.066$).

[c]Value within row different than other value ($P < 0.02$).

[d]Value within column different than other values ($P < 0.01$).

[e]Value within column different than other values ($P < 0.05$).

of indispensable amino acids showed a highly significant increase ($P < 0.01$) in total liver pyridine nucleotide concentration as compared to the value obtained for rats fed the basal diet (979 µg./g. vs. 658 µg./g.). When 0.1% L-tryptophan was added to the diet containing the indispensable amino acids without leucine the liver pyridine nucleotide concentration of the rat again was significantly higher ($P < 0.05$) than values obtained for rats fed the basal diet (1209 µg./g. vs. 880 µg./g.). Thus at both levels of tryptophan the removal of leucine from the amino acid mixture resulted in an increased pyridine nucleotide concentration in the rats. These results indicate that the presence of leucine in the amounts as found in corn interferes with the utilization of tryptophan for pyridine nucleotide synthesis.

The pyridine nucleotide concentration of rats fed diets containing corn was lower than the values obtained for the rats fed the tryptophan-supplemented basal diet (731 µg./g. vs. 880 µg./g.). This difference is not significant, but this may be partly due to the large standard errors of the mean. The liver pyridine nucleotide concentration of rats fed the zein diet was also low, similar to the value obtained for the rats fed corn diets. Thus some amino acid in zein may hinder the use of tryptophan for pyridine nucleotide synthesis. When the tryptophan-supplemented mixture of the dispensable amino acids simulating zein was added to the basal diet, the liver pyridine nucleotide concentration of rats fed this diet was also low, similar to the value obtained for rats fed the corn diet. This indicates that one or more of the dispensable amino acids in zein may be involved in

decreasing the utilization of tryptophan for pyridine nucleotide synthesis.

When the pyridine nucleotide concentrations of rats fed the various diets supplemented with tryptophan are compared to the values obtained with rats fed the same diets, but with limited amounts of tryptophan, significant increases in liver pyridine nucleotide concentration occurred in the rats fed the basal diet ($P < 0.066$), the complete mixture of the indispensable amino acids ($P < 0.02$), and the mixture of the indispensable amino acids without leucine ($P < 0.02$). These increases were 222 μg./g., 180 μg./g., and 230 μg./g., respectively. The concentration of pyridine nucleotides in the liver of rats fed the tryptophan-supplemented complete mixture of indispensable amino acids was surprisingly high. The removal of the mixture of dispensable amino acids possibly allowed for the increase in pyridine nucleotide concentration, but this increase was not as great as it was in the rats fed the basal diet or the diet containing the mixture of indispensable amino acids without leucine. Again, it appears that leucine may interfere with the utilization of L-tryptophan for pyridine nucleotide formation in the rat. However, the pyridine nucleotide concentration of rats fed diets containing corn, zein, and the mixture of the dispensable amino acids was not increased significantly, these increases being only 40 μg./g., 77 μg./g., and 49 μg./g., respectively. These latter small increases in pyridine nucleotide concentration suggest that some dispensable amino acid or acids in corn interfere with the utilization of tryptophan for pyridine nucleotide formation. If

this is true, then it is reasonable to believe that the addition of only the indispensable amino acids to the L-tryptophan supplemented basal diet allowed for the increased pyridine nucleotide concentration of rats fed this diet.

The effect of corn, zein, and mixtures of amino acids simulating zein on the liver concentration of NAD in rats is shown in Table IV. In the unsupplemented groups, the addition of corn, zein, the complete mixture of indispensable amino acids, and the mixture of dispensable amino acids simulating zein did not significantly change the liver concentration of NAD from the value obtained with rats fed the basal diet. The addition of the mixture of indispensable amino acids without leucine significantly increased ($P < 0.05$) the NAD concentration above the value of rats fed the basal diet. In rats whose diets were supplemented with tryptophan, again there was a significant increase ($P < 0.01$) in liver NAD concentration of the rats fed the mixture of indispensable amino acids without leucine over the values obtained with rats fed the other diets.

A comparison of the NAD values of the non-supplemented and the tryptophan supplemented groups on each diet shows that the concentration of NAD was significantly increased in the rats fed the tryptophan supplemented diets containing either the complete mixture of the indispensable amino acids ($P < 0.05$) or the mixture of the indispensable amino acids without leucine ($P < 0.01$).

The effect of corn, zein, and mixtures of amino acids simulating zein on the liver concentration of NADH in rats is

TABLE IV

THE EFFECT OF CORN, ZEIN, AND MIXTURES OF AMINO ACIDS SIMULATING ZEIN WITH AND WITHOUT A 0.1% L-TRYPTOPHAN SUPPLEMENT ON LIVER NICOTINAMIDE ADENINE DINUCLEOTIDE (NAD) CONCENTRATION OF RATS

Groups	Diets	No. of Animals	Without Added Tryptophan	No. of Animals	With 0.1% L-Tryptophan
			µg./g.		µg./g.
I and II	Basal	5	457 ± 61[a]	4	507 ± 28
III and IV	Corn	5	437 ± 39	4	502 ± 88
V and VI	Zein	5	456 ± 17	5	472 ± 26
VII and VIII	Indispensable Amino Acids	8	485 ± 20	9	580 ± 37[b]
IX and X	Indispensable Amino Acids Without Leucine	9	556 ± 21[c]	9	695 ± 39[d,e]
XI and XII	Dispensable Amino Acids	5	463 ± 53	5	473 ± 36

[a]Mean ± SE.

[b]Value within row different than other value ($P < 0.05$).

[c]Value within column different than other values ($P < 0.05$).

[d]Value within column different than other values ($P < 0.01$).

[e]Value within row different than other value ($P < 0.01$).

TABLE V

THE EFFECT OF CORN, ZEIN, AND MIXTURES OF AMINO ACIDS SIMULATING ZEIN WITH AND WITHOUT A 0.1% L-TRYPTOPHAN SUPPLEMENT ON THE REDUCED FORM OF LIVER NICOTINAMIDE ADENINE DINUCLEOTIDE (NADH) CONCENTRATION OF RATS

Groups	Diets	No. of Animals	Without Added Tryptophan	No. of Animals	With 0.1% L-Tryptophan
			µg./g.		µg./g.
I and II	Basal	5	204 ± 34[a]	5	365 ± 69[b]
III and IV	Corn	4	272 ± 83	5	218 ± 40
V and VI	Zein	5	190 ± 17	5	252 ± 38
VII and VIII	Indispensable Amino Acids	3	231 ± 43	4	351 ± 15[c]
IX and X	Indispensable Amino Acids Without Leucine	4	426 ± 18[d]	4	515 ± 44[e]
XI and XII	Dispensable Amino Acids	4	269 ± 21	4	322 ± 46

[a]Mean ± SE.

[b]Value within row different than other value ($P < 0.069$).

[c]Value within row different than other value ($P < 0.05$).

[d]Value within column different than other values ($P < 0.01$).

[e]Value within column different than other values ($P < 0.05$).

shown in Table V. In the unsupplemented groups the addition of corn, zein, the mixture of indispensable amino acids and the mixture of dispensable amino acids simulating zein did not significantly change the liver concentration of NADH from the value obtained with rats fed the basal diet. When the tryptophan supplement was added, again, the only significant increase ($P < 0.05$) over the basal value was obtained from rats fed the diet containing the mixture of indispensable amino acids without leucine.

A comparison of the NADH values of the non-supplemented and the tryptophan supplemented groups on each diet reveals that the concentration of NADH was significantly increased in the rats fed the tryptophan-supplemented basal diet ($P < 0.069$) and in the rats fed the complete mixture of indispensable amino acid diet ($P < 0.05$). Although there was an increase in the concentration of NADH of rats fed the mixture of indispensable amino acids without leucine, the increase was not statistically significant.

The results of this study indicate that one or more dispensable amino acids of zein interfere with liver pyridine nucleotide formation in the rat. These data also clearly show that the omission of leucine from diets containing the complete mixture of indispensable amino acids results in a highly significant increase in liver pyridine nucleotide concentration in the rat. These findings lend support to the ideas of Belavady *et al.* (5) who suggested that leucine interferes with the utilization of tryptophan.

CHAPTER V

SUMMARY

The effect of corn, zein, the complete mixture of indispensable amino acids simulating zein, the mixture of indispensable amino acids simulating zein without leucine, and the dispensable amino acids simulating zein on the utilization of L-tryptophan for pyridine nucleotide synthesis was studied in the rat. The parameters measured were growth and liver pyridine nucleotide concentration. The addition of corn or the complete mixture of the indispensable amino acids to a niacin-free 6% casein basal diet significantly increased ($P < 0.02$ and $P < 0.01$ respectively) the growth of rats fed these diets over that obtained with rats fed the basal diet. The addition of zein markedly depressed growth, but there was no significant growth difference found between rats fed the mixture of indispensable amino acids without leucine or the dispensable amino acids simulating zein and the basal diet. However, when a 0.1% L-tryptophan supplement was added to the various diets, there was a significant increase ($P < 0.02$, $P < 0.01$, and $P < 0.02$ respectively) in the growth of rats fed the diets containing corn, the indispensable amino acid without leucine, and the dispensable amino acids. The addition of a tryptophan supplement did not significantly change the growth of rats fed the other diets.

In groups with limited tryptophan, the addition of corn, zein, the complete mixture of the indispensable amino acids simulating zein,

and the dispensable amino acids simulating zein to the basal diet did not affect the total pyridine nucleotide concentration of the rats fed these diets. However, rats fed the diet in which leucine was omitted from the mixture of indispensable amino acids showed a highly significant increase ($P < 0.01$) in total liver pyridine nucleotide concentration.

In the rats fed the various experimental diets supplemented with 0.1% L-tryptophan, the group fed the mixture of the indispensable amino acids without leucine again showed a significant increase ($P < 0.05$) in liver pyridine nucleotide concentration as compared with the pyridine nucleotide concentration of rats fed the tryptophan-supplemented basal diet. The pyridine nucleotide concentration of rats fed diets containing corn, zein, and the mixture of dispensable amino acids was lower than the values obtained for the tryptophan-supplemented basal diet.

When the total pyridine nucleotide concentrations of rats fed the various diets supplemented with tryptophan are compared to the values obtained with rats fed the same diets but with limited amounts of tryptophan, significant increases in liver pyridine nucleotide concentration occurred in the rats fed the basal diet ($P < 0.066$), the complete mixture of indispensable amino acids ($P < 0.02$), and the mixture of the indispensable amino acids without leucine ($P < 0.02$). However, the pyridine nucleotide concentration of rats fed diets containing corn, zein, and the mixture of the dispensable amino acids was not increased significantly.

These data indicate that one or more dispensable amino acids in zein as well as the amount of leucine in zein interfere with the utilization of tryptophan for pyridine nucleotide synthesis.

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